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Introduction

Our research in the first year has focused on the creation of nanoparticles designed for hyperthermic tumor ablation via magnetic field heating, and the development of equipment for magnetic field generation to effect this ablation. In the area of nanoparticle fabrication, we have changed our focus from metallic nanoparticles to superparamagnetic analogs. This change was made to avoid the high frequencies and energy fluxes required for metallic systems. In our synthetic studies, we have developed particles that are stable and water soluble. In terms of equipment, we have discovered that the magnetic field generator described in the proposal was not powerful enough to obtain rapid heating of particle solutions. We are currently working with MSI Industries to develop coils to be used with their 5 kW induction heating instrument.

Body

We have taken a two-prong approach to developing nanomaterials for magnetic field-mediated tumor ablation: nanoparticle fabrication and the development of equipment for generating alternating magnetic for use in these studies. Overarching these two goals is the issue of effective heating using readily available instrumentation. In our original proposal, we planned to use inductive heating of gold nanoparticles through production of eddy currents in the metallic core by alternating magnetic fields. To heat small gold particles (1.5nm), frequencies in the gigahertz range are required. As reported by Hamad-Schifferli *et al.*, an output power of 4W is required to achieve a local temperature increase of 13°C (from 22°C to 35°C).[1] Although adequate for dehybridization of short complementary oligonucleotides, this minimal degree of heating is insufficient for hyperthermia or tumor ablation applications. After discussions with our Co-PI Siegfried Ingvesson, it became clear that achieving the temperature increases needed for ablation using gold particles would be both difficult and expensive for simple cell culture studies. More significantly, the creation of generators suitable for *in vivo* studies and eventual biomedical applications would be prohibitively expensive, if they were even accessible.

We have examined a variety of options that would allow us to use lower frequencies. The most promising approach appears to be the use of hysteretic heating of superparamagnetic nanoparticles.[2] The primary hurdle for this approach is the ability to produce reasonably monodisperse nanoparticles that can be functionalized with the functionality required for our proposed targeted therapy.

Fabrication of Superparamagnetic Nanoparticles

Our synthetic studies have focused on iron oxide (Fe_2O_3) particles synthesized via the method developed by Alivasatos.[3] In previous studies, we have found that branched diols provide effective capping ligands for these particles.[4] This approach has two distinct advantages over other methodologies such as dextran coating. First, these particles are reasonably monodisperse (Figure 1), making their behavior more predictable and reproducible. Second, this strategy provides a reasonable method for attaching targeting functionality.

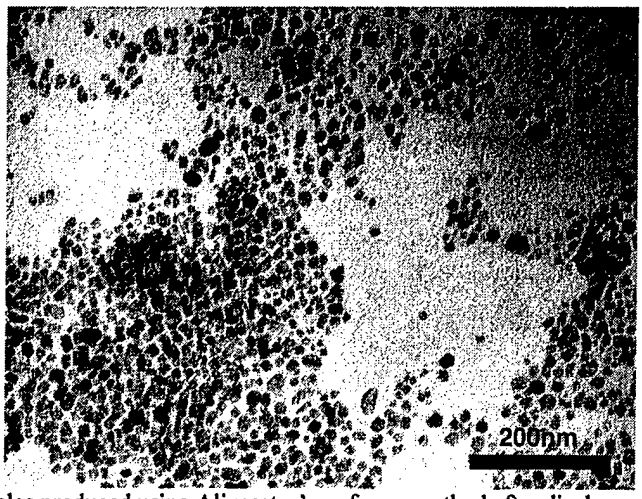
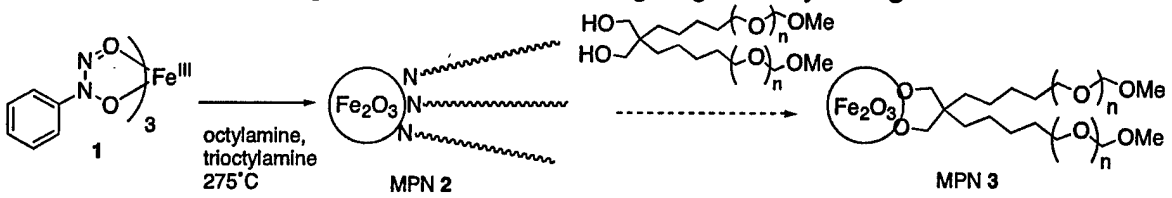


Figure 1. Fe_2O_3 nanoparticles produced using Alivasatos' cupferron method after displacement with diol functionality

In our current studies, we are extending these studies to biocompatible PEGylated systems (Scheme 1) that will hopefully be water soluble and stable in aqueous media. If these systems are successful, it will be straightforward to attach the targeting antibody through the same diol tether.



Scheme 1. Fabrication of MPN 2 via Alivasatos' cupferron method, and proposed formation of biocompatible particles 3 using pegylated sidechains.

Magnetic Field Generation

Concurrent with our synthetic studies, we are developing instrumentation for generating sufficient magnetic field flux for our planned ablation strategy. For these studies, we have used commercially-available dextran-coated iron oxide nanoparticles. Using instrumentation available at UMass, we were able to get modest (<7°C) heating. While this provided some level of cell killing (presumably heat-induced apoptosis, Figure 2), we were unable to get the level of heating required for ablation. For the past three months we have been working with MSI industries to adapt their metalforming equipment to our purposes. After considerable efforts at coil design, we have been able to achieve >20 °C temperature increases over a few seconds in model solutions. We are expecting delivery of the final instrument at the end of June 2004; funds were re-allocated from our personnel budget to pay for this obviously needed equipment.

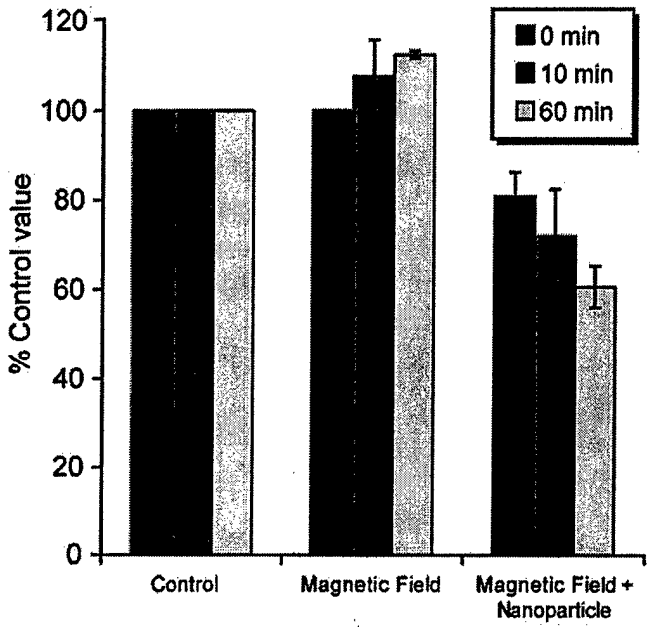


Figure 2. Magnetic field (frequency, 450kHz; amplitude, 80A/m) was applied to cultured MCF-7 cells in the presence or absence of dextran-coated iron oxide nanoparticles (~100ug/80,000 cells). Cells were preincubated (+/- nanoparticles) for 1hr at 37°C in serum-free media to allow for cell-particle association. Cell viability was assayed by tetrazolium salt (MTT) metabolism and reported as percentage of untreated control. Experiments were performed in triplicate.

Key Research Accomplishments

To date, our key accomplishments are in the development of an effective heating strategy that uses readily available equipment that we have adapted for our purposes. We are thus poised to do our proposed cell culture and in vivo experiments once we have worked out the synthesis of our nanoparticles.

Reportable Outcomes

None to date

Conclusions

We have overcome the first hurdle in our research, namely developing a strategy that allows extremely rapid localized heating using an alternating magnetic field. Our goal for the coming year is to integrate this methodology with our synthetic studies. In the meantime, we will continue our cell culture studies using commercially available nanoparticles, which will provide some insight into strengths and limitations of our method.

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